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A Scoping Review: Metabolomics Signatures Associated with Animal and Plant Protein Intake and Their Potential Relation with Cardiometabolic Risk

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Lépine Gaïa, Hélène Fouillet, Didier Rémond, Jean-François Huneau, François Mariotti, and Sergio

Polakof

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4 **Authors:** Gaia Lépine^{1,2}, Hélène Fouillet², Didier Rémond¹, Jean-François Huneau²,
5 François Mariotti², Sergio Polakof¹

6 **Author affiliations:**

7 1 : Université Clermont Auvergne, INRAE, UMR 1019, Unité Nutrition Humaine, Clermont-
8 Ferrand, 63000, France

9 2 : Université Paris-Saclay, AgroParisTech, INRAE, UMR PNCA, 75005, Paris, France

10 **Corresponding author:**

11 Dr. Sergio Polakof

12 INRAE, UMR 1019, UNH, F-63000 CLERMONT-FERRAND

13 Tel: 33 (0) 4 73 62 48 95; Fax: 33 (0) 4 73 62 46 38; e-mail: sergio.polakof@inrae.fr

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21 **Abbreviations:**

22 AA: Amino acids, AAA: Aromatic amino acids, aMD: alternate Mediterranean Diet, AP: Animal
23 proteins, BCAA: Branch-Chained Amino Acids, BMI: Body Mass Index, CVD: Cardiovascular
24 diseases, DASH: Dietary Approach to Stop Hypertension, FIB: Food Intake Biomarker, HDI:
25 Healthy Diet indicator, HEI: Healthy Eating Index, 2-HB: 2-hydroxybutyrate, 3-HIB: 3-
26 hydroxyisobutyrate, MD: Mediterranean diet, NND: New Nordic Diet, PAGln:
27 Phenylacetylglutamine, PP: Plant proteins, PP-to-AP ratio: plant to animal protein ratio, S_c:
28 overall contribution score, S_D: study design contribution score, S_M: metabolite score, S_P: dietary
29 contribution score TCA: Tricarboxylic acid, TMA: Trimethylamine, TMAO: Trimethylamine-N-
30 oxide, T2D: Type 2 Diabetes

31 **ABSTRACT**

32 The dietary shift from animal protein (AP) to plant protein (PP) sources is encouraged for both
33 environmental and health reasons. For instance, PP are associated with lower cardiovascular
34 and diabetes risks compared to AP, although the underlying mechanisms mostly remain
35 unknown. Metabolomics is a valuable tool for globally and mechanistically characterizing the
36 impact of AP and PP intake, given its unique ability to provide integrated signatures and
37 specific biomarkers of metabolic effects through a comprehensive snapshot of metabolic
38 status. This scoping review is aimed at gathering and analyzing the available metabolomics
39 data associated with PP and AP-rich diets, and discussing the metabolic effects underlying
40 these metabolomics signatures and their potential implication for cardiometabolic health.

41 We selected 24 human studies comparing the urine, plasma or serum metabolomes
42 associated with diets with contrasted AP and PP intakes. Among the 439 metabolites reported
43 in those studies as able to discriminate AP and PP-rich diets, 46 were considered to provide a
44 robust level of evidence, according to a scoring system, especially amino acids (AA) and AA-
45 related products. Branch-chained amino acids (BCAA), aromatic amino acids (AAA),
46 glutamate, short-chain acylcarnitines and trimethylamine-N-oxide (TMAO), which are known
47 to be related to an increased cardiometabolic risk, were associated with AP-rich diets, whereas
48 glycine (rather related to a reduced risk) was associated with PP-rich diets. TCA cycle
49 intermediates and products from gut microbiota AAA degradation were also often reported, but
50 the direction of their associations differed across studies.

51 Overall, AP and PP-rich diets result in different metabolomics signatures, with several
52 metabolites being plausible candidates to explain some of their differential associations with
53 cardiometabolic risk. Additional studies specifically focusing on protein type, with rigorous
54 intake control, are needed to better characterize the associated metabolic phenotypes and
55 understand how they could mediate differential AP and PP effects on cardiometabolic risk.

56

57 **Teaser text:**

58 The metabolomics signatures associated with animal and plant protein-rich diets were
59 reviewed to better understand the metabolic effects of animal and plant protein and their impact
60 on cardiometabolic health.

61 **Keywords:** amino acids; biomarkers; cardiovascular diseases; diabetes; dietary patterns;
62 legumes; meat; metabolites; Mediterranean diet; nutrimentalomics; vegetable proteins

63 **Statement of significance:** This work is the first review gathering and analyzing the available
64 metabolomics data associated with diets with contrasted animal and plant protein intakes in
65 humans and discussing the metabolic effects underlying these metabolomics signatures and
66 their potential implication for cardiometabolic health.

67

68 **INTRODUCTION**

69

70 The transition towards more plant-based diets is encouraged with the perspective of moving
71 towards more sustainable diets (1). The shift within protein sources is one key aspect of this
72 transition and dietary guidelines tend to a lower intake of animal protein (AP) sources, more
73 specifically processed and red meat, and a higher intake of plant protein (PP) sources such as
74 whole grains, legumes, nuts and seeds, for improving both human (2–5) and planetary health
75 (6–9). From the standpoint of human health, AP intake has been related with increased risk of
76 several diseases. For instance, consumption of processed meat has been rated as
77 carcinogenic to humans by the International Agency for Research on Cancer especially
78 because of its link with colorectal cancer (10) while more recent evidence suggests low
79 certainty and small effect size (11). AP intake has also been adversely associated with
80 metabolic syndrome related-outcomes (12–18) and with higher risk (and PP with lower risk) or
81 death from type 2 diabetes (T2D) and cardiovascular diseases (CVD)(19–25). Based on these

82 recent and emerging evidence, the present review will specifically focus on the effects of AP
83 and PP on cardiometabolic health.

84 The contrasting effects of AP and PP on cardiometabolic health could be mediated by different
85 factors, such as the “protein package” (i.e. the nutrients and compounds present in protein-
86 rich foodstuff and associated to protein intake) and the overall diet quality associated to AP
87 and PP food sources intake (26–30). The hypothesis that differences in intrinsic amino acid
88 (AA) composition between PP and AP could contribute to their differential effects on
89 cardiometabolic health has also been proposed (29,31). Indeed, AP have a higher content in
90 essential AA such as branched-chain AA (BCAA) and aromatic AA (AAA), while PP have a
91 higher content in non-essential AA such as arginine and glutamate (see **Figure 1**, (32)). Some
92 of these AA were reported as being strongly associated with cardiometabolic health (33–36).

93 In recent years, metabolomics has developed significantly in the field of nutritional sciences in
94 which its application is now often referred to as nutrimetabolomics (37). One active orientation
95 in this field is the search for food intake biomarkers (FIB) (38,39), which are promising tools to
96 evaluate the exposure to food intake by alleviating the usual caveats of dietary assessment
97 obtained from self-reported questionnaires (40). The nutrimetabolomics research also aims at
98 untangling the physiological effects of dietary patterns, foods and nutrient consumption through
99 the discovery of effect biomarkers revealing the impact of a specific nutritional effector on a
100 given physiological function (effect biomarkers).

101 Although several hypotheses have been proposed, the mechanistic bases for the effects of AP
102 and PP on cardiometabolic health are still widely unknown. A metabolomics-based strategy
103 could therefore point to novel hypotheses leading to better understanding of the molecular
104 processes involved. Interestingly, although the association of AP and PP intake with
105 cardiometabolic health has been extensively discussed (29,31,41), only one cross-sectional
106 study has specifically investigated the differences in the plasma metabolome associated with
107 AP and PP intake (42), highlighting the gap in knowledge between metabolomics signatures
108 and protein source consumption in the context of cardiometabolic risk. Thus, this review aims

109 at gathering and analyzing the available data on the metabolomics signatures associated with
110 PP and AP intake to better understand their metabolic effects, and more specifically their
111 impact on cardiometabolic health.

112

113 **CURRENT STATUS OF KNOWLEDGE**

114 **STUDIES AND METABOLITES SELECTION USING A DEDICATED SCORING SYSTEM**

115

116 Given that very few data on the metabolites associated with AP and PP intake are available in
117 pre-clinical models, we focused our analysis on human studies (both observational and
118 interventional) in which a metabolomics approach was applied to plasma, serum or urine
119 samples. The evidence strictly specific to AP and PP metabolomics signatures is scarce (42),
120 but there is more evidence available regarding the metabolites associated with broader dietary
121 changes. After an extensive review of the literature, we retained a subset of studies in which
122 we were able to determine that protein type was an important factor differentiating the diets
123 under study (**Figure 2**).

124

125 Overall, 3 types of dietary comparisons were identified. Firstly, the comparison of omnivores
126 with vegetarians and vegans is likely to reflect a strong contrast in AP and PP intakes. For
127 instance, in Western countries, the proportion of AP in total protein intake is around 70% in
128 omnivores (12,27,43–45), against 30% in vegetarians (46–48). Secondly, although, little
129 quantitative data is available regarding AP and PP intake differences associated to healthy
130 dietary patterns such as the Mediterranean diet (MD), Healthy Eating Index (HEI) or Dietary
131 Approach to Stop Hypertension (DASH), the metabolites associated with these dietary patterns
132 are likely to be associated with AP or PP intake as those dietary patterns contain a higher
133 plant-based fraction (see **Supplemental Table S1** for detailed description of the dietary pattern

134 adherence scores reported across selected studies). For example, the MD is likely to be
135 associated to a higher PP intake than a typical western diet as it includes a high consumption
136 of legumes, nuts and whole grains and a relatively low proportion of AP, corresponding to a
137 moderate consumption of fish, poultry and dairy foods and a low consumption of red meat
138 products (49). Thirdly, the comparison of metabolites associated with dietary patterns
139 constructed a posteriori by cluster analyses can be of relevance when these patterns are
140 closely related to AP or PP food groups, though the actual AP and PP intake differences might
141 be difficult to quantify.

142 Overall, 24 references met the above criteria and were considered in this review: 16
143 observational studies (42,50–64) and 8 clinical trials (65–72) (**Table 1, Table 2**). Regarding
144 the analytical approach used, 16 references were MS-based (42,51,52,55–60,62,63,66–
145 68,71,72) and 8 were NMR-based (50,53,54,61,64,65,69,70). Among the 8 clinical trials, 3
146 compared omnivore vs vegetarian diets or meals (65,70,72), 2 investigated the effects of the
147 MD (69,71) and the remaining 3 measured the effects of the New Nordic Diet (NND) (rich in
148 typical northern vegetables, whole grains, legumes and fish) (66–68).

149 All observational studies had a cross-sectional design and the inter-group differences across
150 the metabolome are rather expected to result from a long-term exposure to the diets compared.
151 One study directly compared AP and PP intake (42), 6 compared omnivores with vegetarians
152 and vegans (50–54,64), 6 compared groups according to adherence to defined dietary patterns
153 (55–60) and 3 according a posteriori (data-driven) dietary patterns (61–63). While most studies
154 were conducted on healthy participants, 8 of them included participants with cardiometabolic
155 risk factors (42,58,66–69,71,72).

156 To account for the differences in study design and for the disparities expected in AP and PP
157 intakes, we built a scoring system that balances the potential contribution of each study to
158 improve our ability to identify metabolites associated to AP and PP-rich diets. Briefly, for each
159 study, we applied a study design contribution score (S_D) based on the levels of evidence
160 defined by the Oxford Centre for Evidence-Based Medicine (73) and a dietary contribution

161 score (S_P) based on the expected differences in protein type intake and available dietary data
162 (**Figure 2**). The highest S_P values were attributed to studies investigating directly the
163 association of the metabolome with AP and PP intake, followed by studies comparing either
164 vegetarians or vegans with omnivores, different adherence levels to healthy dietary patterns,
165 and finally a posteriori (data driven) dietary patterns. Then, for each study, an overall
166 contribution score (S_C) was calculated by averaging S_D and S_P , which ranged from 0.33 to 0.84
167 across studies (**Supplemental Table S2 and S3**). For each selected study, we retrieved the
168 discriminating metabolites (see **Supplemental text** for additional explanations). Then, we
169 summed the S_C of each study reporting a specific metabolite to obtain an overall metabolite
170 score (S_M). For a given metabolite, a high S_M can be the result of its presence in numerous
171 studies with a low S_C or its presence in only a few studies but with a high S_C . We finally
172 distinguished a short-list of metabolites being associated with AP and PP-rich diets with a
173 higher level of evidence among those having the highest S_M values, by applying a S_M cut-off
174 value of 1.51 which represent 3-fold of 60% of the maximum S_C attributed (0.84), so as to
175 highlight the metabolites cited in at least 3 studies of relatively good relevance.

176

177 **OVERALL HIGH HETEROGENEITY IN THE REPORTED METABOLITES BUT**

178 **CONVERGENCE ON AA METABOLISM**

179

180 Across the selected studies, 439 metabolites were reported as discriminant between diets with
181 varying AP and PP contents, with a mean S_M of 0.89. We summed the S_M values of all
182 metabolites within one metabolite class to estimate its overall contribution to the global
183 metabolomics signature obtained (**Figure 3**). Metabolites related to protein and AA metabolism
184 had the highest contribution (high number of metabolites and high mean S_M), followed by
185 phosphatidylcholines (high number of metabolites), and then by metabolites of energy and
186 carbohydrate metabolism and acylcarnitines (moderately high number of metabolites and a

187 moderately high mean S_M). Taken together, the different lipid classes contributed to a high
188 proportion (45%) to the overall metabolomics signature.

189 Moreover, only 46 of these 439 discriminant metabolites were judged to have a sufficient level
190 of evidence, using a S_M cut-off value of 1.51 that ensures concordance of metabolite detection
191 across studies of good relevance (see **Table 3**). Among them, 14 (30%) were AA, which
192 highlights their important role in the metabolomics signatures associated with AP or PP-rich
193 diets. This short-list also contained other important endogenous metabolites such as
194 tricarboxylic acid (TCA) cycle intermediates or creatine, creatinine and choline, mostly
195 provided by the dietary intake of animal products. This short-list contained few lipids although
196 they were very abundant previously, probably because of the high variability of this particular
197 family that may explain the lack of convergence between studies. Moreover, the high
198 contribution of AA-related molecules to metabolomics signatures can be interpreted in terms
199 of biomarkers of exposure and/or effects. Indeed, such metabolomics signatures could reflect
200 the difference in the intrinsic AA composition of AP and PP (Figure 1), which intakes were
201 contrasted in the diets compared, but are probably not simple protein FIB, since circulating AA
202 levels are usually not considered to directly reflect AA dietary intake (48). They more likely
203 constitute effects biomarkers, either of overall dietary patterns with contrasted protein sources
204 or more specific to protein type intake as differences in AA intake could lead to modifications
205 in protein and AA metabolism that in turn become visible in the metabolome.

206

207 **REPLICATION OF ALREADY KNOWN FOOD INTAKE BIOMARKERS**

208

209 Across the selected studies, many identified metabolites were already known as potential FIB.
210 For instance, several metabolites known for their association with animal products and meat
211 consumption were found associated with high AP diets.

212 Creatine and its degradation product creatinine have been proposed as total meat FIB,
213 creatine being more reliable than creatinine which excretion is closely related to individual
214 muscle mass (74). Both metabolites had amongst the highest S_M values (4.11 and 4.24
215 respectively) and were often associated with high AP diets (42,51,53,54,57,64,65,69,70).
216 Likewise, 3-methylhistidine ($S_M=2,06$) has been proposed as a total meat and poultry FIB (74)
217 and was associated with diets with a higher proportion of AP (50,69,72).

218 Metabolites associated with plant food intake were also reported to be associated with PP-rich
219 diets. For instance, hippurate ($S_M=2,39$), a metabolite formed through endogenous glycine
220 conjugation of benzoic acid but also through gut microbiota degradation of dietary polyphenols
221 (75,76), was positively associated with diets with a high proportion of PP (50,59,66), to one
222 exception in a MD intervention study (69).

223

224 **EFFECT OF AP AND PP-RICH DIETS ON METABOLISM**

225

226 **Association between metabolomics signatures and cardiometabolic risk**

227 Several studies included in this review conducted mediation analyses between metabolomics
228 signatures derived from diets with different proportions of AP and PP and cardiometabolic risk
229 or risk factors. In an analysis of the PREDIMED baseline data, adherence to MD and the
230 related metabolomics signature (60 metabolites) were associated with a lower CVD incidence
231 (58). Furthermore, the association between the metabolomics signature and CVD risk
232 remained significant when adjusting for MD adherence. In addition, the association between
233 MD score and CVD was 37% mediated by the metabolomics signature, in accordance with
234 another analysis in which the metabolomics signature derived from MD adherence also
235 mediated 37% of the negative association between MD adherence and HOMA-IR (57).

236 These mediation analyses suggest that some of the discriminating metabolites between diets
237 with contrasted AP and PP levels may contribute to explain the differential effects of these
238 diets on cardiometabolic risk, by acting as some of the causal effectors.

239

240 **BCAA, AAA and acylcarnitines**

241 BCAA (leucine, isoleucine and valine) and AAA (such as tyrosine and phenylalanine) are
242 present in high quantities in AP (Figure 1). In association with certain short-chain
243 acylcarnitines, they have been consistently reported together in the metabolic signatures
244 associated with several metabolic diseases and are discussed together.

245 All BCAA and AAA were in the short-list of metabolites consistently identified by relevant
246 studies (with high S_M values ranging between 4,83 and 2,05, Table 3) and were most
247 consistently associated with high-AP diets, this association being repeated in observational
248 and at least one interventional study for each metabolite (with the exception of tyrosine,
249 reported only in observational studies). They were positively associated with omnivore diets
250 (50–53,64,70), with the Average Danish Diet compared to the NND (67), and negatively
251 associated with PP-to-AP ratio and PP intake (42) and with MD adherence (57,71). In a dietary
252 cluster analysis, the 3 BCAA, tyrosine and phenylalanine were also associated with the
253 Western dietary pattern but not the vegetarian one (62). Conversely, an association with PP-
254 rich diets was observed in only 2 cases: phenylalanine was positively associated with the PP-
255 to-AP ratio (42) and leucine was positively associated with the 2 MD intervention groups of the
256 PREDIMED study (69).

257 Short-chain acylcarnitines, especially C3 and C5 acylcarnitines, are byproducts of BCAA
258 mitochondrial oxidation (77). It is therefore not surprising that short-chain acylcarnitines have
259 been reported to be associated with high-AP diets. This was the case in particular for
260 acylcarnitine C4 and C2 (present in the short-list of Table 3 with high S_M values of 2.44 and
261 2.18, respectively), which were consistently reported in both observational and interventional

262 studies (42,51,58,61,62,65,68,71). In additional analyses, urine acylcarnitine C2 was positively
263 associated with meat intake, further confirmed by a comparison across meat intake percentile
264 groups (61). C3-acylcarnitine ($S_M=1.29$) was positively associated with AP-rich diets in a cross-
265 sectional study comparing vegans and non-vegans (51) and an MD randomized trial (71).
266 Finally C5-acylcarnitine ($S_M=0.90$) was also associated with AP-rich diets, since it was
267 associated with a western diet dietary cluster (62) and found at higher levels in habitual non-
268 vegans (51).

269 BCAA, AAA and short-chain acylcarnitines were associated with several cardiometabolic risk
270 factors. In the Fenland study, adherence to MD was inversely associated with HOMA-IR, and
271 the integration of acylcarnitines in the prediction model resulted in an 11% attenuation of the
272 association, suggesting their implication in the initiation and progression of insulin resistance
273 (57). Further, in the metabolomic profiling of Asian Indians living in the USA (62), one of the
274 identified clusters of metabolites included BCAA, phenylalanine, and tyrosine, C2 and C5
275 acylcarnitines and was positively associated with high animal product intake dietary pattern
276 and several cardiometabolic risk markers (higher fasting insulin, higher HOMA-IR, total
277 cholesterol, triglyceride and fat liver levels).

278 Outside the studies included in this review, there is wealth of examples of associations
279 between metabolic disorders and this group of metabolites. For instance, a PCA component
280 including BCAA, C3 and C5 acylcarnitines, phenylalanine, tyrosine, methionine and
281 glutamate/glutamine was positively associated with obese compared to lean subjects and had
282 a significant linear relationship with HOMA-IR, even after adjusting for the lean versus obese
283 status (36). Since then, elevated plasma BCAA, phenylalanine and tyrosine have been often
284 reported in cardiometabolic related conditions (78–81) and numerous cohort studies (78–
285 80,82–92). Those metabolites also seem to have a predictive value for cardiometabolic
286 disease risk. BCAA, tyrosine, phenylalanine were predictive of higher T2D and CVD risk in
287 prospective cohorts (93–98). Regarding acylcarnitines, while several studies showed an

288 association with CVD (96,99–101), only C5 and C16-acylcarnitines were reported so far as
289 positively associated with increased risk of T2D (94).

290 Several explanations have been discussed to explain the potential causal link of this group of
291 metabolites with cardiometabolic disorders. There is supporting evidence that BCAA overload
292 promotes mitochondrial dysfunction by acylcarnitine accumulation (102). One possible
293 mechanism involves the metabolite 3-hydroxyisobutyrate (3-HIB), a byproduct of valine
294 oxidation that occurs in the mitochondria, but which is also able to leak into the blood circulation
295 (77). In rodents, 3-HIB has been shown to favor fatty acid accumulation in the muscle (103)
296 by leading to acylcarnitine accumulation inside the mitochondria and promoting mitochondrial
297 dysfunction, thought to promote insulin resistance (102). In humans, plasma 3-HIB was
298 significantly associated with a higher risk of T2D, even after adjustment for plasma BCAA and
299 obesity (104). Interestingly, 3-HIB ($S_M=0,58$) was positively associated with habitual meat
300 consumption in the present review (53).

301 The results regarding 2-hydroxybutyrate (2-HB) also strengthen the hypothesis of increased
302 fatty acid oxidation in association with AP intake. In the present review, 2-HB ($S_M=0.94$) was
303 associated with AP-rich diets in 2 cross-sectional studies (63,91). On the other hand, 2-HB has
304 been associated with a higher risk of insulin resistance and T2D in prospective cohorts
305 (105,106). In addition, in an untargeted metabolomics analysis, 2-HB proved to be very
306 discriminant and able to distinguish insulin resistant individuals from insulin sensitive ones
307 (107). Given that the production of 2-HB from α -ketobutyrate (a byproduct of methionine and
308 threonine) requires the oxidation of NADH to NAD⁺, a higher concentration of 2-HB could
309 reflect a higher fatty acid oxidation, known to increase the NADH/NAD⁺ ratio (105,107).
310 Another explanation for the high 2-HB levels could relate to an increase α -ketobutyrate
311 production during the conversion of cystathionine to cysteine, in order to fuel glutathione
312 synthesis under high oxidative stress conditions (107).

313 In conclusion, the consumption of diets richer in AP is associated with higher plasma BCAA,
314 AAA and short-chain acylcarnitines. Higher levels of those metabolites have been observed in
315 individuals with higher cardiometabolic risk factors such as insulin resistance. The causal role
316 of these metabolites in the onset of insulin resistance is not fully established but there is
317 growing evidence of a mechanism involving an overload of mitochondrial beta-oxidation
318 capacity induced by high BCAA circulating levels and concomitant acylcarnitine mitochondrial
319 accumulation. Therefore, BCAA, phenylalanine, tyrosine and short-chain acylcarnitines are
320 good candidates to explain the association between AP intake and increased cardiometabolic
321 risk.

322

323 **Glycine, glutamine and glutamate**

324 Non-essential AA glycine, glutamine and glutamate, which were also in the short-list of
325 metabolites consistently identified by relevant studies (with high S_M values ranging between
326 4,03 and 2,41, Table 3), are known for their implication in cardiometabolic health.

327 Glutamate ($S_M=2.41$) was positively associated to AP-rich diets in 4 cross-sectional studies: it
328 was negatively associated with MD adherence (57,58), positively associated with a dietary
329 pattern high in meat and low in vegetable (63) and with omnivores compared to vegetarians
330 (52). However, fish-eaters and vegetarians had higher plasma glutamate than omnivores in
331 the EPIC Oxford cohort (51), and glutamate was positively associated with vegetarian meals
332 and negatively with vegan meals and omnivore meals in one interventional study (70), a finding
333 that cannot be interpreted in terms of association with AP and PP meal intake.

334 Plasma or serum glutamine ($S_M=2.50$) was positively associated with PP-rich diets in 3 cross-
335 sectional studies comparing habitual omnivores and vegetarians or vegans (51,53,64). This
336 was consistent with its lower presence in the urine of high meat eaters in a 2-week clinical trial
337 (65).

338 Glycine ($S_M=4.03$) was found very consistently to be positively associated with PP-rich diets
339 both in observational and interventional studies. Plasma or serum glycine was positively
340 associated with habitual vegetarianism and veganism (50,51,53,54) and with the NND
341 intervention group in a Danish clinical trial (67). In urine, it was further associated with the MD
342 intervention groups in the PREDIMED study (69) and with a dietary pattern with a high
343 proportion of plant-foods (61). Additional findings, which were not obtained with a
344 metabolomics approach, also reported a positive association between plasma glycine and PP
345 sources intake (48,108–110), despite glycine intake usually being lower in diets richer in PP.
346 Consistent with these findings, an analysis of the EPIC-Potsdam cohort found a negative
347 association between red meat consumption and plasma glycine (111). The association
348 between red meat consumption and T2D risk was 30% mediated by plasma glycine,
349 suggesting that glycine could be a potential mediator of the differential effect of AP and PP on
350 cardiometabolic health.

351 Indeed, a meta-analysis of 47 case-control studies showed decreased serum glycine and
352 glutamine and increased glutamate in patients with obesity and metabolic syndrome compared
353 to healthy controls (112), in accordance with several cross-sectional analyses (78,79). On the
354 other hand glutamate was rather positively associated to cardiometabolic risk factors
355 (78,80,113). A consistent set of studies also showed that the circulating levels of these AA are
356 predictive of cardiometabolic disease risks. Plasma glycine and glutamine were negatively
357 associated with T2D risk while plasma glutamate was associated with higher risk in two meta-
358 analyses of prospective cohorts (94,101). Glutamate was also associated with a higher risk of
359 CVD in several prospective cohorts (114–116), while glutamine-to-glutamate ratio and glycine
360 were linked to a lower risk (114,117,118). Interestingly, one of the studies selected in this
361 review demonstrated both a negative association between plasma glutamate and MD and a
362 positive association with CVD risk (58).

363 Several mechanisms have been discussed to explain the positive effects of glycine and
364 glutamine on cardiometabolic health, including their implication in glutathione synthesis for the

365 regulation of oxidative stress (119,120). In addition, in endothelial cells, the production of
366 ammonia from glutamine to glutamate leads to increased heme degradation and byproduct
367 production, i.e. the bile pigments biliverdin and bilirubin, and carbon monoxide (CO), known to
368 be involved in oxidative stress and inflammatory regulation (119). Glutamine could also be
369 indirectly implicated in endothelial cell function and nitric oxide synthesis, a regulator of blood
370 flow and pressure (121). In the intestine, glutamine is converted into glutamate and citrulline,
371 the latter being exported into the circulation and further converted by the kidney into arginine,
372 the substrate for nitric oxide synthase (119,122). Citrulline ($S_M=1.74$) was indeed a discriminant
373 metabolite across 3 studies included in this review, with a positive association with high-PP
374 diets in a cross-sectional study comparing omnivores with vegetarians and vegans and in a
375 MD randomized trial (51,71) and a negative association with MD adherence level in another
376 cross-sectional study (57). Other potential explanatory mechanisms include the anti-
377 inflammatory effect of glutamine given its capacity to stimulate macrophage differentiation into
378 the M2 anti-inflammatory type (123).

379 In contrast, although the mechanisms by which glutamate could contribute to an increased
380 cardiometabolic risk are not well known, its toxicity to the β -cells in a high glucose context has
381 been proposed as a possible explanation to the increased T2D risk observed (124,125).

382 To conclude, there is strong evidence that glycine is associated with PP-rich diets and
383 decreased cardiometabolic risk. Glutamine and glutamate seem to be associated with PP and
384 AP-rich diets, respectively, although with a less conclusive level of evidence than for glycine.
385 Their associations with cardiometabolic health are contrasted, the positive association of
386 glutamine being backed-up by more mechanistic explanations than the negative one for
387 glutamate.

388

389 **TCA cycle intermediates**

390 In our analyses, several TCA cycle intermediates, such as citrate ($S_M=3.09$), isocitrate
391 ($S_M=1.51$), succinate ($S_M=1.99$), malate ($S_M=1.15$) and cis-aconitate ($S_M=1.10$), were
392 repeatedly associated with diets with different proportions of AP and PP (Table 3). However,
393 there was no clear trend regarding the direction of those associations. For instance, malate
394 was positively associated with PP intake and the PP-to-AP ratio (42), but it was negatively
395 associated with MD, aHEI and DASH score (59) in two observational studies. Glycolysis end
396 products such as lactate ($S_M=1.55$) and pyruvate ($S_M=0.84$) were reported to be either
397 associated to vegetarian meal consumption or to the average Danish diet in another nutritional
398 intervention (67,70).

399 The TCA cycle is essential in energy metabolism, and the modulation of its related metabolites
400 is indicative of effects on the central metabolism. However, further investigations are needed
401 to clarify the direction of the association with either AP or PP-rich diets. In addition, there is not
402 yet enough data to understand whether and how these metabolites are linked to
403 cardiometabolic health and if they could be meaningful in the explanation of the physiological
404 effects of AP and PP intake.

405

406 **EFFECTS OF AP AND PP-RICH DIETS ON GUT MICROBIOTA METABOLISM**

407

408 Amongst the studies included in this review, the three that specifically focused on gut
409 microbiota showed differences in activity and composition between vegan, vegetarian and
410 omnivore diets (54), and according to MD adherence (52,71).

411 Plasma and urine metabolomics data also suggested that AP and PP-rich diets induce a
412 differential activity of gut microbiota, with several gut-derived metabolites being involved
413 (42,50,61,65,66,68,69,71).

414

415 **TMAO**

416 Trimethylamine-N-oxide (TMAO) comes from two different sources. TMAO is produced by the
417 liver's conversion of trimethylamine (TMA), which itself results from the degradation by the gut
418 microbiome of several dietary precursors such as choline, phosphatidylcholine, betaine,
419 carnitine, dimethylglycine and ergothioneine (126). Plasma TMAO is also increased by the
420 consumption of fish (in which it is naturally present) and has therefore been proposed as a
421 short-term FIB of this food category (74).

422 TMAO was in the short-list of metabolites consistently identified by relevant studies (with a
423 high S_M value of 3,79, Table 3) and was associated to AP-rich diets in both observational and
424 interventional studies. Urinary TMAO was hence positively associated with omnivore diet (50),
425 a dietary pattern with a high meat intake (61), the control group of the PREDIMED trial (69)
426 and the high meat group of another nutritional intervention (65). The only exception was
427 observed in the New Nordic trial where higher TMAO levels were found for the diet with a
428 higher proportion of PP, but probably because this diet was also richer in fish (66,68). In
429 addition, similar findings were obtained in an interesting 16-week cross-over randomized trial
430 where participants consuming 2 plant-based alternatives per day instead of 2 serving of meat,
431 while maintaining similar intake for all food categories, had lower serum TMAO levels overall
432 (127). Similar results were found in another well-controlled trial, where a red meat diet led to
433 higher plasma TMAO levels than a white meat diet and a non-meat diet richer in PP (128).

434 The differences in TMAO concentration between dietary protein sources could result from a
435 higher intake of TMAO precursors with AP-rich diets, but also depend on other factors, such
436 as gut microbiota composition, hepatic enzyme activity converting TMA to TMAO, and TMAO
437 renal excretion (129). The higher TMAO levels associated with AP-rich diets could also indicate
438 higher cardiometabolic risk associated with this type of protein sources, without it necessarily
439 being implicated causally.

440 TMAO plasma levels have indeed been positively associated with all-cause mortality, major
441 adverse cardio- and cerebro-vascular events (130–132) and T2D (133). In addition, TMAO has

442 also been linked to several risk factors such as hypertension (134), elevated BMI (135) and
443 systemic inflammation, as assessed by C-reactive protein (136).

444 Research is still active to understand the mechanistic bases of these associations and whether
445 they are causal (126,129). In addition, these findings still need to be reconciled with the
446 association of TMAO with fish intake, considered beneficial to cardiometabolic health (137), in
447 particular cardiovascular health (138). Despite these limitations, these recent findings suggest
448 that TMAO could be a promising biomarker of cardiometabolic risk. Therefore, since we found
449 in this review that TMAO is associated with AP-rich diets, it can be considered as a potential
450 mediator or biomarker of the higher cardiometabolic risk associated with higher consumption
451 of this protein type.

452

453 **Products of AAA gut microbiota degradation**

454 Although most proteins are digested and absorbed in the small intestine, a small fraction can
455 reach the colon and be fermented by the microbiota. Across the selected studies, several
456 microbiota metabolites derived from AAA degradation have been reported as discriminant of
457 AP and PP-rich diets.

458 Phenylacetylglutamine (PAGln), a product of phenylalanine, was in the short-list of the
459 metabolites consistently identified by relevant studies ($S_M=2.43$). It was inversely associated
460 in cross-sectional studies with the PP-to-AP ratio (42) but positively associated with a dietary
461 cluster with a low meat intake (61), where secondary analyses reported a positive association
462 between its urinary concentration and vegetable intake. Results are conflicting regarding
463 PAGln association in MD randomized trials as it was reported to be either positive (69) or
464 negative (71).

465 Although no conclusion can be drawn regarding the direction of its association with AP or PP-
466 rich diets, this metabolite is worth mentioning as emerging evidence suggests its association
467 with increased CVD risk (139,140).

468 Tryptophan can also be catabolized by specific bacteria of the gut into indole propionic acid,
469 indole lactic acid and indole acetic acid (145). These 3 tryptophan-bacteria derived byproducts
470 were reported across the articles selected in this review, but the direction of the association
471 with either AP or PP-rich diets was not clear. Plasma indole-3-propionate ($S_M=1,41$) was
472 associated with high PP intake (42) and to adherence to MD in an 8-week dietary intervention
473 (71). In the same trial, oxindole-3-acetic acid ($S_M=0,71$) was also positively associated with MD
474 adherence. On the other hand, urinary indole-3-acetic acid glucuronide ($S_M=0,71$) (66) and
475 plasma indole-3-lactic acid ($S_M=0,71$) (68) were positively associated with the Average Danish
476 Diet that predominantly includes AP sources.

477 Interestingly, these indole derivatives are thought to exert a positive effect on the intestinal
478 physiology (improved intestinal immune response, regulation of the intestinal barrier and
479 gastrointestinal motility and stimulation of glucagon-like-peptide 1 secretion), but also on the
480 host, by promoting direct anti-inflammatory effects. The evidences regarding these
481 mechanisms, reviewed extensively recently (141,142), are mostly derived from in vitro and
482 animal studies and additional research is needed to confirm these findings in human studies.

483 Indoxyl sulfate ($S_M=1,41$), another metabolite of tryptophan, and p-cresol ($S_M=2,08$) derived
484 from tyrosine (143) were generally associated with AP-rich diets across the selected studies.
485 Plasma indoxyl sulfate was negatively correlated to PP intake (42) and adherence to the MD,
486 as was p-cresol (71). However, p-cresol was also positively associated with the MD
487 intervention groups of the PREDIMED study (69). Both are well known uremic toxins with
488 harmful effects in chronic kidney disease (144).

489 Together, these findings highlight the possible implication of these microbial AAA degradation
490 products on cardiometabolic health, with either an increase in CVD risk for PAGIn or beneficial

491 effects for indole derivatives. Because they were reported as discriminant by several studies
492 included in this review, they are likely to be associated with diets with contrasted protein type
493 intake but the direction of those associations needs to be clarified.

494

495 **LIMITS**

496

497 Several limitations should be mentioned regarding this review. To the best of our knowledge,
498 only one cross-sectional study specifically investigated the metabolomics signature associated
499 to the nature (AP or PP) of the protein intake (42). To reduce this knowledge gap, we also
500 considered both interventional and observational studies in which AP and PP intakes were
501 likely to differ between groups, even if the magnitude of these differences were often not
502 precisely quantified. With the implementation of a dedicated scoring system for weighting each
503 study's contribution according to the extent of the expected differences in protein intake
504 between its groups and the level of evidence expected from its study design, we were able to
505 account for these between-studies differences in our global analysis. However, we could not
506 fully compensate for the initial lack of dietary data, and the design quality was only roughly
507 estimated since no validated tool is available to assess all those criteria on the same scale.

508 Overall, the metabolites reported here were discussed in relation to AP or PP intake, but this
509 interpretation remains subject to caution because of several additional reasons. Firstly, AP and
510 PP are not consumed alone but as whole foods within diets: the "whole food package" or
511 "protein package" not only contains dietary proteins but also other nutrients and compounds,
512 such as fiber, vitamins, unsaturated or saturated fats, that may contribute to the physiological
513 effects observed (145). Secondly, the consumption of AP and PP sources is closely associated
514 with overall diet quality. For instance, fish consumption is associated with a healthier dietary
515 pattern, with higher vegetable intake and lower meat and alcohol intake (26). PP itself is

516 associated with nutrient adequacy and diet quality (27,28), and the nutrient clusters associated
517 with AP and PP could be important mediators of long term health effects (29,30).
518 Consequently, findings obtained in a specific dietary context cannot be readily generalized to
519 other populations with a different dietary environment.

520 It should also be kept in mind that analyses grouping all AP or PP sources together are not
521 representative of the large variability of their metabolic effects that differ according to the
522 specific sources, especially within AP sources. A set of meta-analyses investigating the effects
523 of food group intakes on health outcomes has shown an increased risk of all-cause mortality,
524 CVD, hypertension, T2D and overweight associated with red meat and processed meat while
525 fish and dairy intake were associated with decreased risks (146–150).

526 On the other hand, to date, there is no available analytical tool able to describe the entire
527 human metabolome. Therefore, our understanding of the modulation of the metabolome
528 remains limited to the detected and annotated molecules.

529

530 **MAIN FINDINGS AND PERSPECTIVES**

531

532 Overall, the consumption of AP and PP-rich diets have been associated with distinct
533 metabolites, confirming the relevance of nutrimentalomics in exploring the complex
534 interactions between dietary protein sources and health outcomes.

535 Despite considerable heterogeneity in the metabolites identified and the level of evidence,
536 several of them were good candidates to explain the effects of AP and PP intake on
537 cardiometabolic health (**Figure 4**). Plasma BCAA, AAA and short-chain acylcarnitines were
538 positively associated with AP-rich diets: they are of particular interest because they have often
539 been reported together and have been repeatedly associated with cardiometabolic risk factors
540 in multiple design settings. This metabolic signature might be an early marker of muscle

541 lipotoxicity, and partially explain the positive association between AP intake and higher
542 cardiometabolic risk. AP-rich diets also induce higher TMAO urinary levels, which have been
543 linked to higher cardiovascular risks, although causality remains to be established. In contrast,
544 circulating levels of glycine and glutamine were positively associated with PP-rich diets, and
545 both have been proposed to exert beneficial effects on cardiometabolic health. On the other
546 hand, glutamate has been associated with AP-rich diets and increased cardiometabolic risk.

547 To overcome the limits discussed previously regarding the difficulty of interpreting differences
548 in diet in terms of variations of protein source in the studies published, additional studies with
549 a specific focus on protein type, detailed dietary data and strict intake control are needed: both
550 observational studies including a large number of participants and closely describing the
551 protein and AA intake, and randomized, controlled trials investigating the specific effects of AP
552 and PP intake on plasma and urinary metabolomes. Such detailed studies are required to
553 better characterize the metabolites associated to PA and PP intake, and to understand how
554 they can impact cardiometabolic risk.

555

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559 All the authors have read and approved the final manuscript.

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FIGURES

Figure 1: Amino acid compositions of the most common animal protein and plant protein sources, expressed as a percentage of egg amino acid content.

Values for each category of protein sources were calculated as the average amino acid content of 3 or 4 common foods within the category as follows, based on USDA database (32): Cereals: white wheat flour, white rice, corn flour. Pulses: Red kidney beans, lentils, tofu, chickpeas. Nuts: almonds, walnuts, peanuts. Dairy: whole milk (cow), plain yogurt (cow), Gruyere cheese. Terrestrial meat: beef, pork, chicken. Fish: cod, salmon, trout. Egg: only the values corresponding to whole hen egg were used. Data are presented as a percentage of egg amino acid content per 100g of total amino acid.

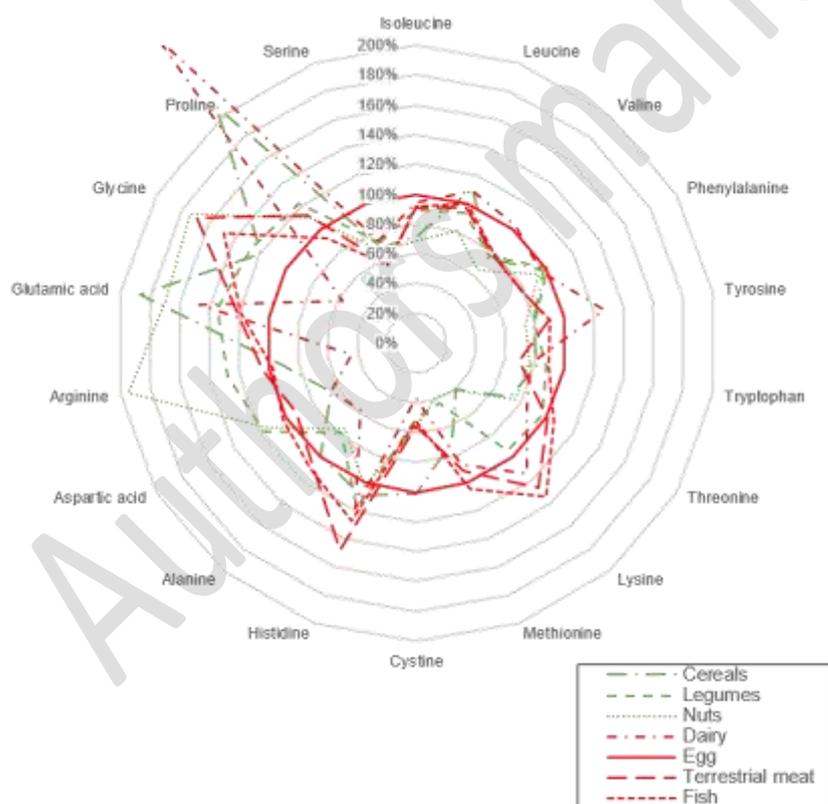


Figure 2: Description of the methodology applied for study selection and data analysis to identify metabolites associated with diets with contrasted AP and PP intake.

AP: Animal proteins, DASH: Dietary Approach to Stop Hypertension, HEI: Healthy Eating Index, PP: Plant proteins

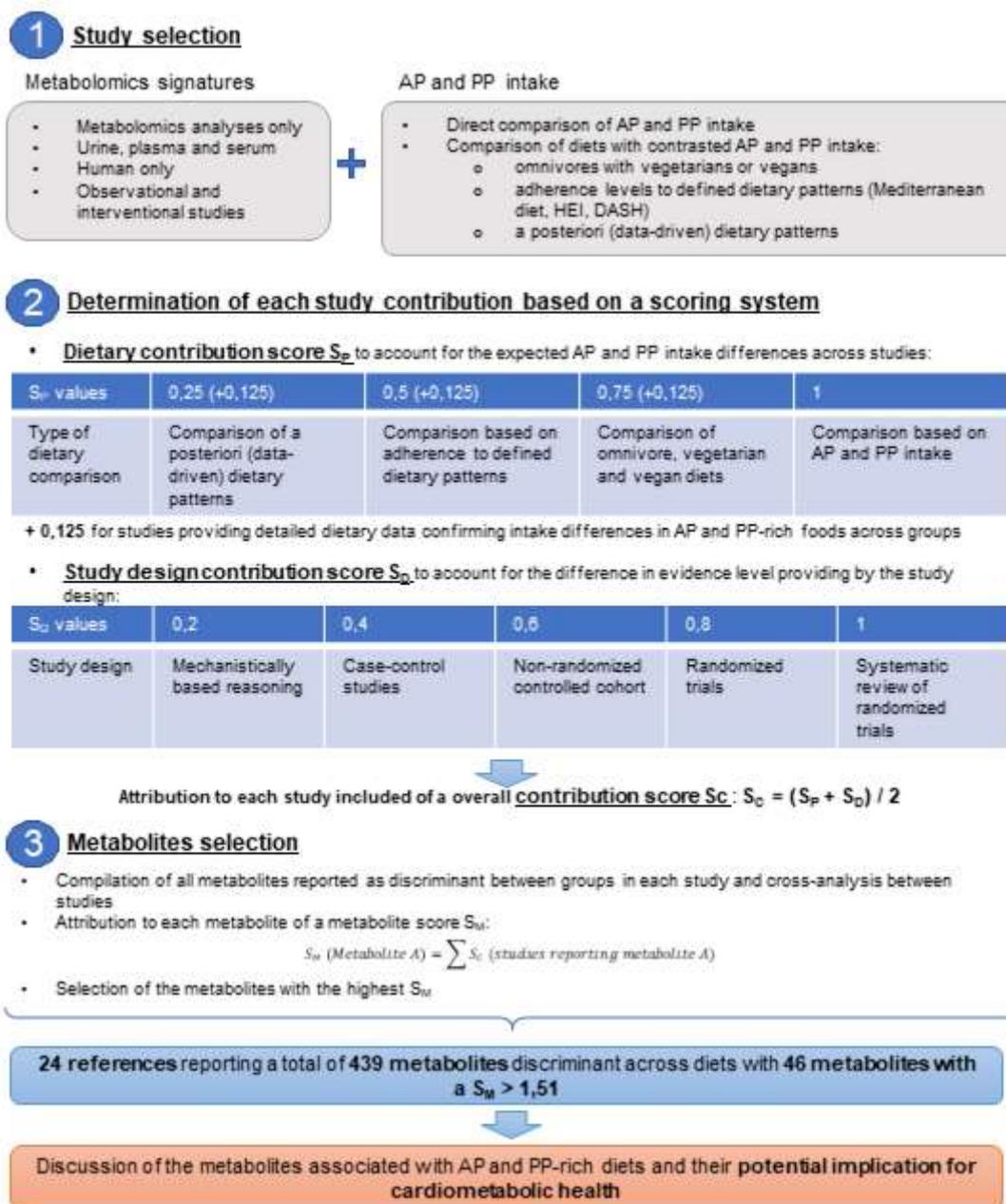


Figure 3: Contribution of each metabolite class to the overall metabolomics signature associated to AP or PP-rich diets, weighted by overall metabolite score.

Sum S_M is the relative weigh attributed to a metabolite class and calculated as the sum of S_M values of each metabolite reported within this class; n is the number of metabolites reported per class; mean S_M is the average S_M value within one metabolite class; % MS represent the

percentage of the Sum S_M value obtained by MS-based studies as opposed to NMR-based studies.

AP: animal protein, PP: plant protein, S_M : metabolite score,

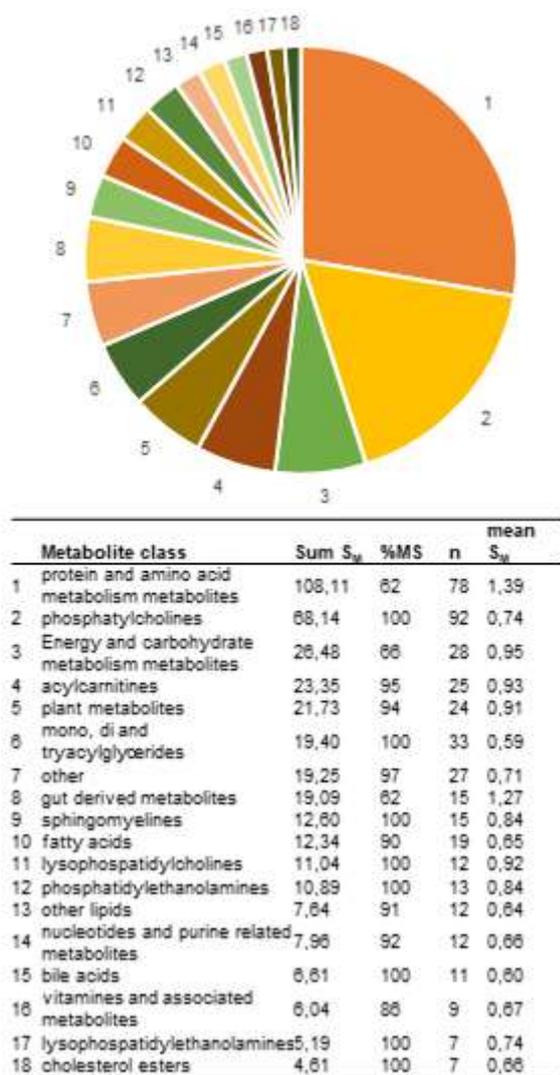


Figure 4: Main metabolites discriminant between animal protein (AP) and plant protein (PP) rich diets and their potential implication in cardiometabolic risk.

AP and PP-rich diets are associated with distinct urine, plasma and serum metabolites in humans. BCAA (leucine, isoleucine, valine), tyrosine, phenylalanine, glutamate, short-chain acylcarnitines and gut-derived TMAO were associated with AP-rich diets while glycine and glutamine were associated with PP-rich diets. Regarding their potential implication in

cardiometabolic risk, metabolites associated with AP-rich diets have been shown to be associated with higher risk while metabolites associated with PP-rich diets have been associated with lower risk.

BCAA: branch-chained amino acids, TMAO: trimethylamine-N-oxide

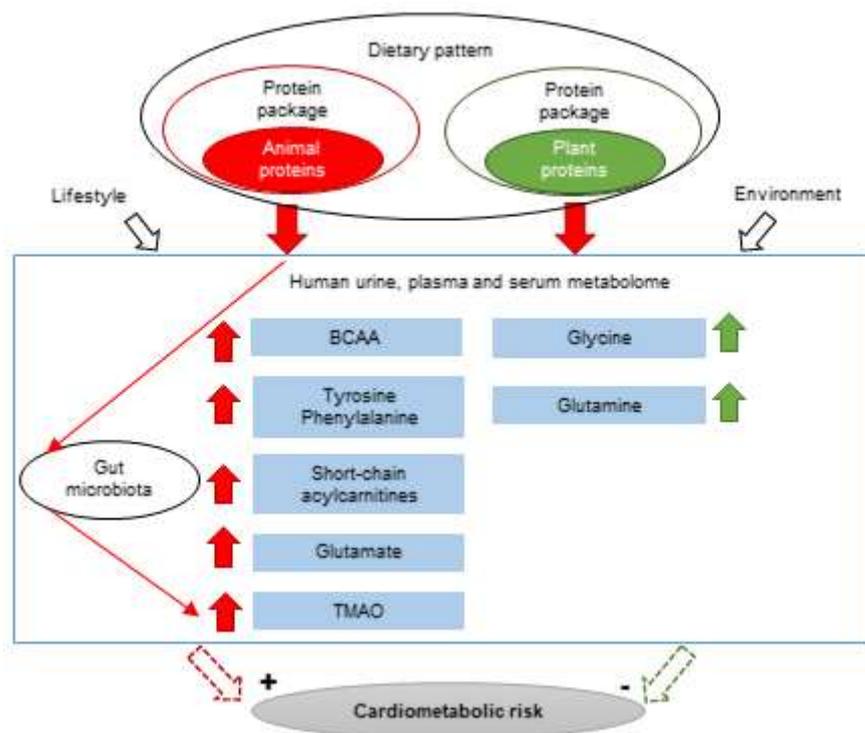


Table 1: Description of the observational studies comparing the metabolites associated to diets with contrasted AP and PP intakes through a metabolomics approach¹

Ref	Type of diet compared (<i>Food intake assessment method</i>)	Study design and cohort (<i>Country</i>)	Population studied	n	Metabolomics analysis	Biofluid	S _c
Comparison based on AP and PP intake							
(42)	Stratification of subjects by AP and PP intake (<i>FFQ</i>)	Cross-sectional analysis of the baseline of PREDIMED (Prevención con Dieta Mediterránea) study according to protein type intake (<i>Spain</i>)	Men and women with high CVD risk (T2D or at least 3 cardiovascular risk factors)	1833	Untargeted LC-MS	Plasma	0.70
Comparison of omnivores, vegetarians and vegans							
(50)	Habitual omnivores Habitual lactovegetarians Diet followed for at least 5 year (<i>None</i>)	Cross-sectional analysis according to vegetarian status (<i>China</i>)	Healthy men and women.	180	Untargeted NMR	Urine	0.58
(51)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet (<i>FFQ</i>)	Cross-sectional analysis of the EPIC (European Prospective Investigation into Cancer and Nutrition)-Oxford cohort according to vegetarian status (<i>UK</i>)	Healthy men only, between 30 and 49 years old	379	Targeted 145 metabolites MS/MS BIOCRATES kit	Plasma	0.58
(52)	Omnivores Lacto-ovo-vegetarians Vegans Diet followed for a at least 6 months (<i>3-day dietary records</i>)	Cross-sectional analysis according to vegetarian status (<i>China</i>)	Healthy men and women	36	Targeted 132 metabolites GC-MS	Serum	0.58

¹ aHEI: alternate Healthy Eating index, aMD: alternate Mediterranean Diet, AP: animal protein, CVD: Cardiovascular diseases, DASH: Dietary Approach to Stop Hypertension, FFQ: Food Frequency Questionnaire, HDI: Healthy Diet indicator, HEI: Healthy Eating Index, MD: Mediterranean Diet, PP: plant protein, S_c: score of study contribution (see text for additional explanations), T2D: Type 2 Diabetes

(53)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet (4-day weighted dietary records)	Cross-sectional analysis according to vegetarian status (Sweden)	Healthy men and women	120	Untargeted NMR	Serum	0.58
(54)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet (4-day weighted dietary records)	Cross-sectional analysis according to vegetarian status (Sweden)	Healthy men and women	118	Untargeted NMR	Urine	0.58
(64)	Omnivores Pesco-vegetarians Lacto-ovo-vegetarians Vegans No criteria on the duration of adherence to the diet (FFQ)	Cross-sectional analysis of the EPIC (European Prospective Investigation into Cancer and Nutrition)-Oxford cohort according to vegetarian status (UK)	Healthy men only, between 30 and 49 years old	286	Targeted NMR	Serum	0.58
Comparison based on adherence to defined dietary patterns							
(55)	HEI-2010 aMD HDI Baltic Sea Diet Score (FFQ)	Cross-sectional analysis of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study according to dietary pattern (Finland)	Healthy men aged between 50-69 years and smokers	1336	Untargeted LC-MS	Serum	0.45
(56)	aMD aHEI HEI-2015 DASH (FFQ)	Cross-sectional analysis of the Cancer Prevention Study-II Nutrition Cohort according to dietary pattern (USA)	Healthy postmenopausal women	1367	Untargeted LC-MS	Serum	0.51
(57)	MD (FFQ)	Cross-sectional analysis of the Fenland Study according to dietary pattern and cardiovascular disease risk factors (UK)	Healthy men and women	1080 6	Targeted 188 metabolites BIOCRATES kit	Plasma	0.45

(58)	MD (FFQ)	Cross-sectional analysis according to dietary pattern and longitudinal analysis according to CVD risk <i>Test cohort:</i> PREDIMED study, median follow-up=4.8 years (Spain) <i>Validation cohorts:</i> Nurse Health Study I, median follow-up=22.3 years Nurse Health Study II, median follow-up=15.3 years Healthy women Health Professionals study, median follow-up=17.6 years (USA)	Test cohort: Men and women with high CVD risk (T2D or at least 3 cardiovascular risk factors) Validation cohort: healthy men and women	Test cohort n=1856 Validation cohort n=6868	Untargeted LC-MS	Plasma	0.45
(59)	aHEI DASH MD (FFQ)	Cross-sectional analysis of the Framingham Off Springs Study according to dietary pattern (USA)	Healthy men and women	2284	Targeted LC-MS	Serum	0.45
(60)	HEI-2015 aHEI-2010 DASH aMD (FFQ)	Cross-sectional analysis of the Atherosclerosis Risk in Communities Study according to dietary pattern (USA)	Healthy men and women	3862	Untargeted LC-MS and GC-MS	Serum	0.45
Comparison of a posteriori (data-driven) dietary patterns							
(61)	Comparison of 2 dietary patterns: - A pattern with more meat products, less vegetables - A pattern with more vegetables and less meat products. (3-day food records)	Cross-sectional analysis (Ireland)	Healthy men and women	160	Untargeted NMR	Urine	0.36
(62)	Comparison of 2 dietary patterns: - "western / nonvegetarian": poultry + fish + red meat + coffee + pizza + alcohol	Cross-sectional analysis of the MASALA (Metabolic Syndrome and Atherosclerosis in South Asians Living in America) pilot study (USA)	Healthy men and women	145	Targeted number of metabolites not specified (above 60)	Serum	0.33

	- "vegetarian": rice + legumes, sweet beverages + snacks (FFQ)				MS		
(63)	Comparison of 2 dietary patterns: - High meat, low vegetable - Low meat, high vegetable (FFQ)	Cross-sectional analysis (Swiss)	Healthy men and women	89	Targeted 87 metabolites in plasma and 103 in urine GC-MS + LC-MS/MS	Urine and plasma	0.36

Table 2: Description of the interventional studies comparing the metabolites associated to diets with contrasted AP and PP intakes through a metabolomics approach¹

Ref	Study design	Type of diet compared	Population studied (Country)	n	Metabolomics analysis	Biofluid	Sc
(65)	Cross-over 15 days of controlled diet	-High meat -Low meat -Vegetarian diet	healthy men (UK)	12	Untargeted NMR	Urine	0.78
(66)	Parallel 6-month intervention A large proportion of food provided through a dedicated shop + dietary counselling	-NND: high content of vegetables, fruits, whole grains, nuts, fish and seafood products -Control: Average Danish Diet: close to actual Danish food consumption	Men and women with high waist circumference (Denmark)	145	Untargeted LC-MS	Urine	0.71
(67)	Parallel 6-month intervention A large proportion of food provided through a dedicated shop + dietary counselling	-NND: high content of vegetables, fruits, whole grains, nuts, fish and seafood products -Control: Average Danish Diet: close to actual Danish food consumption	Men and women with high waist circumference (Denmark)	145	Untargeted GC-MS	Plasma	0.71
(68)	Parallel 6-month intervention	-NND: high content of vegetables, fruits, whole grains, nuts, fish and seafood products	Men and women with high waist circumference (Denmark)	145	Untargeted LC-MS	Plasma	0.71

¹AP: animal protein, MD: Mediterranean diet, NND: new Nordic Diet, PP: plant protein, PP-to-AP ratio: plant protein to animal protein ratio, Sc: score of study contribution (see text for additional explanations), TMAO: trimethylamine-N-oxide

	A large proportion of food provided through a dedicated shop + dietary counselling	-Control: Average Danish Diet: close to actual Danish food consumption					
(69)	Parallel 1 to 3-year intervention Dietary counselling with nuts and olive oil provided	-Low fat diet: instruction to reduce all type of fats and recommendations according to American heart association -MD and olive oil supplementation (1L/week) -MD and nuts supplementation (30g/day)	PREDIMED study Men and women, non-diabetics but with high cardiovascular risk (Spain)	98	Untargeted NMR	Urine	0.65
(70)	Cross-over Postprandial follow up after the consumption of different test meal (3 hours after meal consumption)	-Omnivore meal -Vegetarian meal -Vegan meal	Healthy men and women (Sweden)	32	Untargeted NMR	Serum	0.84
(71)	Parallel 8-week intervention Combination of dietary counselling and food supply	-MD: induced an increase in PP-to-AP ratio by 2.5 -Control diet: habitual food consumption Isocaloric diets adjusted for each participant	Obese and overweighted men and women (mean BMI = 30) (Italy)	62	Untargeted combined with targeted approach for TMAO, carnitine, choline, creatinine and betaine LC-MS	Urine and Serum	0.71
(72)	Cross-over 4 weeks of controlled diet	-Meat-based diet -Vegetarian diet	Mostly men (94%) with ischemic heart disease (Sweden)	27	Untargeted LC-MS/MS	Plasma	0.84

Table 3: Short-list of metabolites associated with AP and PP-rich diets with a sufficient level of evidence, as defined by a S_M value above 1.51¹

Metabolite	S_M	Associated to AP-rich diets		Associated to PP-rich diets	
		Obs	Int	Obs	Int
Leucine*	4.83	(51,52,62) ^{a,#} (53,64) ^{a,§}	(71) ^{a,#} (70) ^{a,§}		(69) ^{b,§}
Creatinine*	4.24	(51,57) ^{a,#} (53,64) ^{a,§} (54) ^{b,§}	(70) ^{a,§}		(69) ^{b,§}
Valine*	4.16	(42,51,52,62) ^{a,#} # (53,64) ^{a,§}	(70) ^{a,§}		
Creatine*	4.11	(42,51) ^{a,#} (53) ^{a,§}	(70) ^{a,§} (65) ^{b,§}		(69) ^{b,§}
Carnitine	4.05	(42,51,57) ^{a,#}	(72) ^{a,#} (71) ^{b,#} (65) ^{b,§}		
Glycine*	4.03			(51) ^{a,#} (53) ^{a,§} (50,54,61) ^{b,§}	(67) ^{a,#} (69) ^{b,§}
TMAO*	3.79	(50,61) ^{b,§}	(65,69) ^{b,§}		(68) ^{a,#} (66) ^{b,#}
Proline betaine	3.69		(68) ^{a,#} (66) ^{b,#} (69) ^{b,§}	(55,60) ^{a,#}	(71) ^{a,#}
Alanine	3.60	(52,62) ^{a,#}	(67) ^{a,#}	(51) ^{a,#} (64) ^{a,§}	(70) ^{a,§}
Threonine	3.41	(57) ^{a,#}	(67,68) ^{a,#} (70) ^{a,§}	(42) ^{a,#}	
Citrate*	3.09		(67) ^{a,#}	(64) ^{a,§} (50,54) ^{b,§}	(69) ^{b,§}
Isoleucine*	2.76	(52,57,62) ^{a,#} (53) ^{a,§}	(70) ^{a,§}		
Lysine	2.56	(51,52) ^{a,#} (53) ^{a,§}	(70) ^{a,§}		
Lysophosphatidyl- choline a C16:1	2.56	(51,57) ^{a,#}	(72) ^{a,#}	(42) ^{a,#}	
Glutamine*	2.50		(65) ^{b,§}	(51) ^{a,#} (53,64) ^{a,§}	
Proline	2.45	(57,58,62) ^{a,#} (53) ^{a,§}			(69) ^{b,§}
Methionine	2.44	(51,62) ^{a,#}	(70) ^{a,§}	(42) ^{a,#}	
Acylcarnitine C4*	2.44	(42,51,58) ^{a,#}	(68) ^{a,#}		
Phenylacetyl- glutamine*	2.43	(42) ^{a,#}	(71) ^{b,#}	(61) ^{b,§}	(69) ^{b,§}

¹ AP: animal protein, CMPF: 3-Carboxy-4-methyl-5-propyl-2-furanpropanoate, DHA: docosahexaenoic acid, GABA: gamma-aminobutyrate: Interventional studies, Obs: observational studies, PP: plant protein, S_M : metabolite score reflecting the degree of concordance of its identification as a discriminant metabolite between studies of good relevance (see text for additional explanations), TMAO: trimethylamine-N-oxide

* Metabolite discussed in the present review

^a Analysis conducted in plasma or serum

[#] MS-based analysis

[§] NMR-based analysis

^b Analysis conducted in urine

Glutamate*	2.41	(52,57,58,63) ^{a,#}		(51) ^{a,#}	
Hippurate	2.39		(69) ^{b,§}	(59) ^{a,#} (50) ^{b,§}	(66) ^{b,#}
Phenylalanine*	2.31	(62) ^{a,#} (50) ^{b,§}	(67) ^{a,#}	(42) ^{a,#}	
Tryptophan*	2.19	(51) ^{a,#}	(71) ^{a,#}	(57,59) ^{a,#}	
Acylcarnitine C2*	2.18	(62) ^{a,#} (61) ^{b,§}	(71) ^{b,#} (65) ^{b,§}		
Lysophosphatidylcholine a C16:0	2.13	(51) ^{a,#}	(72) ^{a,#}		(68) ^{a,#}
GABA	2.09	(42,52) ^{a,#} (63) ^{b,#}		(58) ^{a,#}	
p-cresol*	2.08		(71) ^{a,b,#}		(69) ^{b,§}
3-methylhistidine	2.06	(50) ^{b,#}	(72) ^{a,#} (69) ^{b,§}		
Tyrosine*	2.05	(51,52,62) ^{a,#} (64) ^{a,§}			
Betaine	2.00		(70) ^{a,§}	(59) ^{a,#}	(71) ^{a,#}
Choline	1.99	(59) ^{a,#}	(70) ^{a,§}	(42) ^{a,#}	
Succinate*	1.99	(52) ^{a,#}		(50) ^{b,§}	(70) ^{a,§}
Taurine	1.93	(53) ^{a,§} (50) ^{b,§}	(65) ^{b,§}		
1-methylhistidine	1.93	(42) ^{a,#} (50) ^{b,§}	(71) ^{a,#}		
Tryptophan betaine	1.88			(55) ^{a,#}	(71) ^{a,b,#}
Citrulline*	1.74	(57) ^{a,#}		(51) ^{a,#}	(71) ^{a,#}
Kynurenine	1.73	(51,58) ^{a,#}		(42) ^{a,#}	
Acylcarnitine C18:2	1.73			(42,51,57) ^{a,#}	
CMPF	1.68			(55,56) ^{a,#}	(71) ^{a,#}
Threonic acid	1.61			(55,56,60) ^{a,#}	(67) ^{a,#}
Acylcarnitine C18	1.60	(42,57,58) ^{a,#}			
Lactate*	1.55		(67) ^{a,#}		(70) ^{a,§}
Lysophosphatidylethanolamine C22:0	1.54		(72) ^{a,#}	(42) ^{a,#}	
DHA	1.54	(64) ^{a,§}		(55,56) ^{a,#}	
Sphingomyelin (d18:2/18:1)	1.54	(51,56,59) ^{a,#}			
Isocitrate*	1.51	(59) ^{a,#} (63) ^{b,#}		(42) ^{a,#}	